Megakaryocyte growth and development factor (MGDF): An Mpl ligand and cytokine that regulates thrombopoiesis

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Abstract

Megakaryocyte growth and development factor (MGDF) is a ligand for the Mpl receptor related to thrombopoietin (TPO). MGDF stimulates megakaryocytopoiesis and thrombopoiesis, and is highly selective to cells bearing the Mpl receptor. Studies done in rodents, nonhuman primates, and humans have confirmed that MGDF can increase platelet counts in normal and chemotherapy- or radiotherapy-treated subjects. Platelet function and physiology remain normal after MGDF administration, with no effect on platelet aggregation. Pegylated recombinant human MGDF (PEG-rHuMGDF) was used clinically with initial success. Cancer patients receiving chemotherapy showed dose-dependent increases in platelet counts and increases in bone marrow megakaryocytes. Clinical development of PEG-rHuMGDF was halted owing to the formation of neutralizing antibodies in some patients and normal volunteers who received the drug. The application of exogenous recombinant Mpl ligands should be explored in the setting of randomized clinical trials and the findings extended to mobilization of CD34* stem cells, ex vivo expansion techniques, and use in platelet abnormalities.

Keywords:
MGDF; thrombopoiesis; recombinant protein; platelet function

Introduction

Hematopoiesis, the orderly proliferation, differentiation, and maturation of blood-forming cells, is controlled by many stimulatory and inhibitory regulators (Figure 1). Endogenous proteins, called hematopoietic growth factors or colony-stimulating factors, are of critical importance in this process. Genes for many of these hematopoietic growth factors have been discovered and cloned.

Human megakaryocyte growth and development factor (MGDF), a ligand for the Mpl receptor related to human thrombopoietin (TPO), stimulates megakaryocytopoiesis and thrombopoiesis in vivo.[1,2] Recombinant human (rHu) MGDF can stimulate megakaryocyte colony formation from a subpopulation of CD34* progenitor cells,[3] and these colonies can give rise in vitro to platelets that are morphologically and functionally normal.[4] Systemic administration of rHuMGDF has been shown to increase peripheral platelet counts in mice,[5] rhesus monkeys,[6] and humans.[7-9]

The biologic properties of MGDF suggest that this cytokine is highly selective to cells that bear the Mpl receptor: CD34+ cells, megakaryocytes, and platelets.[1,10,11] This review will discuss a pegylated form of rHuMGDF (PEG-rHuMGDF), one of the two forms of recombinant Mpl ligand that has been produced by recombinant DNA technology; another form (rHuTPO) is in clinical development. The clinical development of PEG-rHuMGDF was halted in 1998 because of the formation of neutralizing antibodies to MGDF in some patients and volunteers who had received the drug. However, not all recipients develop antibodies: one patient with cyclic thrombocytopenia successfully received PEG-rHuMGDF for almost a year at the initial report[12] without bleeding, formation of antibodies, or other side-effects.

Historical overview: two-stage factor regulation of megakaryocytopoiesis

Theories of megakaryocyte development and platelet production have suggested that before thrombopoiesis, at least two independently regulated stages of megakaryocyte development had to occur involving two distinct regulatory factors. The first factor induced commitment and expansion of megakaryocyte precursors into megakaryocytes, and the second factor directed megakaryocyte maturation, ploidization, and platelet formation. The early-acting factor, termed megakaryocyte colony-stimulating factor (Meg-CSF), was identified by use of the megakaryocyte colony-forming (CFU-Meg) assay.[13] The other factor controlling late-stage megakaryocytopoiesis, thrombopoietin (TPO), could induce platelet production in vivo.[14] Endogenous production of Meg-CSF was considered to be a response to a decrease in megakaryocyte mass. Conversely, TPO production was thought to

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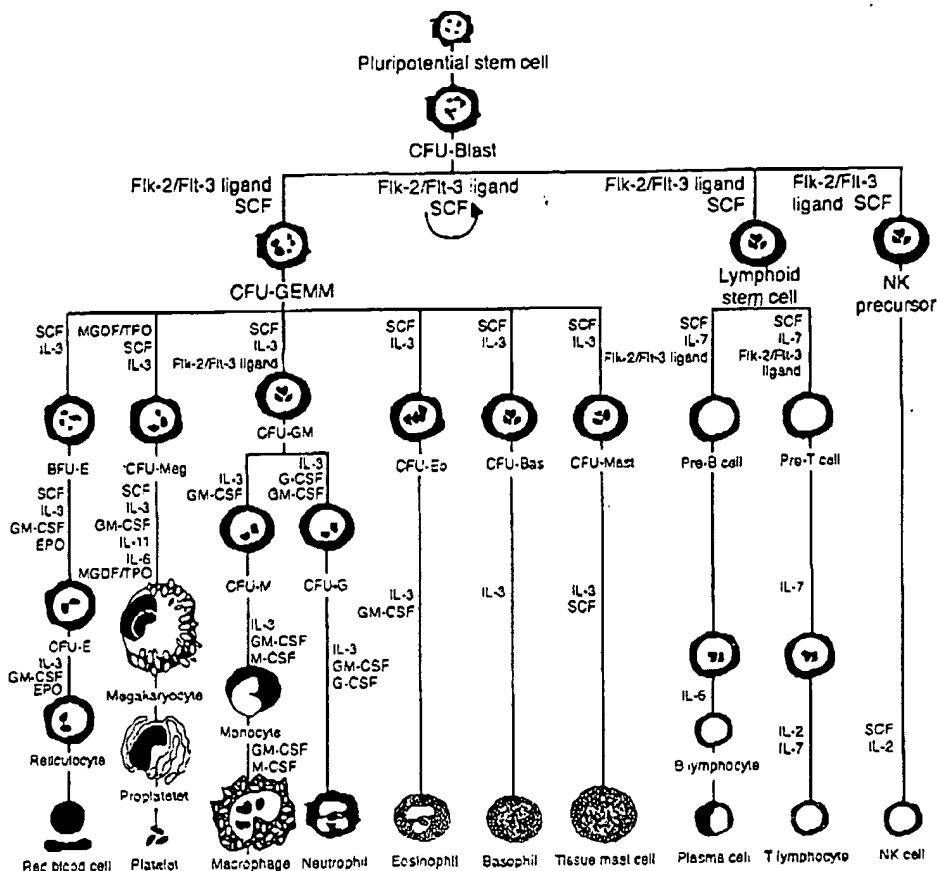
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Pluripotential stem cell

CFU-Blast

Flk-2/Flt-3 ligand SCF   Flk-2/Flt-3 ligand SCF   Flk-2/Flt-3 ligand SCF   Flk-2/Flt-3 ligand SCF

CFU-GEMM   Lymphoid stem cell   NK precursor

SCF IL-3   MGDF/TPO SCF IL-3   SCF IL-3 Flk-2/Flt-3 ligand   SCF IL-3   SCF IL-3   SCF IL-3   SCF IL-7 Flk-2/Flt-3 ligand   SCF IL-7 Flk-2/Flt-3 ligand

BFU-E   CFU-Meg   CFU-GM   CFU-Eo   CFU-Bas   CFU-Mast   Pre-B cell   Pre-T cell

SCF IL-3 GM-CSF EPO   SCF IL-3 GM-CSF IL-11 IL-6 MGDF/TPO   IL-3 GM-CSF   IL-3 G-CSF GM-CSF

CFU-E   CFU-M   CFU-G

IL-3 GM-CSF EPO   IL-3 GM-CSF M-CSF   IL-3 GM-CSF G-CSF   IL-3 GM-CSF   IL-3   IL-3 SCF   IL-7   IL-7

Reticulocyte   Megakaryocyte   Monocyte GM-CSF M-CSF

IL-6   IL-2 IL-7

Proplatelet   B lymphocyte

IL-2 IL-7   SCF IL-2

Red blood cell   Platelet   Macrophage   Neutrophil   Eosinophil   Basophil   Tissue mast cell   Plasma cell   T lymphocyte   NK cell

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be stimulated by a decrease in platelet mass in a feedback-loop mechanism.[15] This theory was based on the observation that substantial Meg-CSF activity has been measured in the plasma or sera of patients with aplastic anemia and in patients or animals after myeloablative therapy, but the activity is nearly absent from the plasma and sera of patients with idiopathic thrombocytopenic purpura. Plasma from patients with idiopathic thrombocytopenic purpura, however, did stimulate platelet production in some animal models. These findings plus other data led to the concept that two separate factors may be responsible for megakaryocyte growth and maturation.

In summary, the traditional theory of the regulation of the megakaryocyte development and platelet formation involves two factors: Meg-CSF and TPO.

## Purification, cDNA cloning, and expression of the Mpl ligand

The existence of one factor that stimulates the production of megakaryocyte precursor cells, megakaryocytes, and platelets was suggested by Keleman et al,[16] and was

demonstrated as an activity several years later in preclinical studies by Odell et al.[17] Souyri et al[18] discovered a new hematopoietic receptor subfamily while studying murine leukemia and oncogenes. This receptor was the product of the gene c-*mpl*, the normal homologue of the newly discovered oncogene v-*mpl*. The gene was discovered to be the transforming factor of a murine myeloproliferative leukemic virus, and was capable of panmyeloid transformation.[18,19] The newly discovered receptor (Mpl) was determined to be the receptor important for the regulation of thrombopoiesis.[20]

In 1992, the observation of Wendling and colleagues that the cytokine receptor Mpl was present on megakaryocytes and platelets provided key information that aids our understanding of this complex system.[19] The earlier observation of the presence of increased Meg-CSF activity in plasma from myelocompromised animals was exploited using this novel growth-factor receptor with standard protein purification methods. A protein was identified almost simultaneously by several different research groups and shown to be the ligand for the Mpl receptor.[1,10,11,21-23] With the tools generated as a result of these efforts, investigation of all aspects of megakaryocyte/platelet development is now possible. In vitro exper-
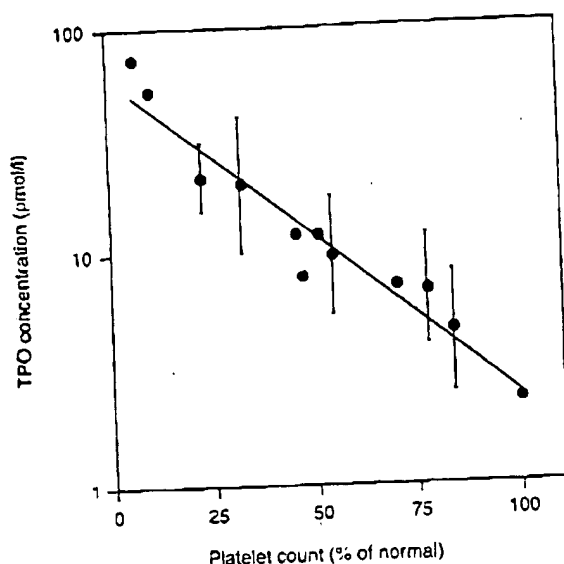
Figure 2
Levels of thrombopoietin (TPO) are inversely and exponentially proportional to platelet count during busulfan-induced thrombocytopenia. (Adapted from reference 26.)
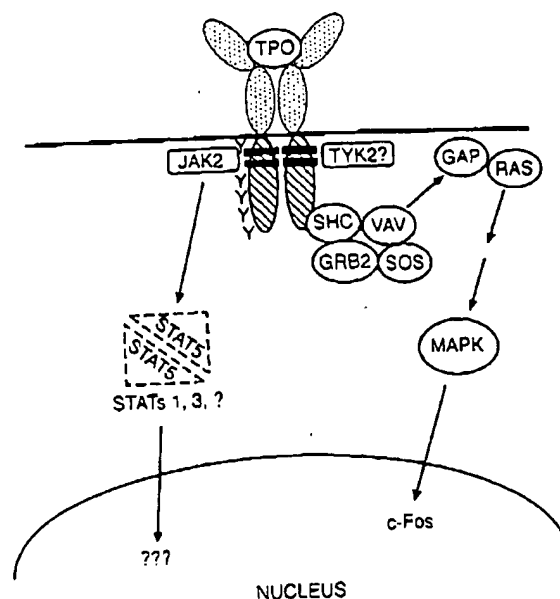
Figure 3
Model of Mpl signal transduction and proposed pathway. (Adapted from reference 31.)

iments have shown that the Mpl ligand is capable of inducing colony formation as well as cytoplasmic and nuclear maturation. The cells stimulated with Mpl ligand can also produce functional platelets in vitro and in vivo. These data confirm that Mpl ligand is capable of all of the functions formerly assigned to either Meg-CSF or TPO. The term 'TPO' has been adopted for the native form of Mpl ligand.

The TPO enzyme-linked immunosorbent assay (ELISA) has shown that plasma concentrations of endogenous TPO in normal subjects are lower than those found in plasma from patients with either aplastic anemia or idiopathic thrombocytopenic purpura (median, 64 pg/ml; $n = 97$).[24] In addition, plasma from patients with aplastic anemia ($n = 22$) contains median concentrations of TPO that are approximately fourfold greater than those obtained in plasma from patients with idiopathic thrombocytopenic purpura ($n = 45$): 723 pg/ml versus 195 pg/ml TPO, and $24 \times 10^9/l$ versus $40 \times 10^9/l$ platelets, respectively. TPO has been shown to be produced by the liver and cleared by the receptors present on platelets and, to a lesser extent, by megakaryocytes. This 'platelet sponge' theory could explain the circulating TPO levels in aplastic anemia and idiopathic thrombocytopenic purpura (Figure 2).[25]

A poly(ethylene)glycol-derivatized, truncated form of recombinant Mpl ligand (PEG-rHuMGDF) was produced and evaluated in clinical trials. This protein demonstrated all the biological activity and in vitro and in vivo characteristics of native Mpl ligand. PEG-rHuMGDF combined the enhanced stability of truncated forms of Mpl ligand with the longer half-life seen with the full-length form.[27-29]

The role of the Mpl ligand and other cytokines in the hematopoietic system

The primary role of human TPO or the related Mpl ligand, MGDF, is to support megakaryocytopoiesis. The mechanism of action by which this occurs is through binding of the ligand to the Mpl receptor, which induces tyrosine phosphorylation.[30] Tyrosine phosphorylation appears to activate secondary signaling pathways, such as the JAK2 signal transduction pathway (Figure 3). Cell-line studies suggesting phosphorylation of additional molecules, including TYK2, STAT3, and STAT5, have been reported, but unique signal transduction pathways for megakaryocyte development have not been identified and are being investigated.[32-34]

The role of other cytokines in the development of the megakaryocytic lineage has been investigated.[35-42] Other megakaryocytic cytokines may play a secondary role in endogenous cytokines may play a secondary role in maintaining basal platelet counts. Evidence for this role comes from work by investigators who developed a TPO knockout transgenic mouse model in which the mice do not produce endogenous TPO.[43] This non-lethal genetic alteration resulted in a 85% reduction in platelet counts (Figure 4).[44] This finding suggests that other cytokines, possibly interleukin (IL)-3, may support the residual low platelet count. The role of these secondary cytokines in the early phases of megakaryocyte progenitor development and in disease states of thrombocytosis, and their relationship (if any) to endogenous TPO, are unclear.

In addition to the effects on platelets, secondary pleiotropic cytokines, such as IL-3, IL-6, and IL-11, have

Figure 4
Platelet count in c-Mpl-deficient mice. (Adapted from reference 44.)

demonstrated a capacity to produce potential adverse reactions in humans. IL-1 has been associated with transient increases in heart rate, low-grade fevers, rigors,[45] and a dose-limiting hypotension.[46] In a phase 1 trial of rHuIL-3, doses of 5 μg/kg or more were not tolerated because such doses produced grade III fever and headache.[47] Other effects have been reported with rHuIL-6 at doses of 1 μg/kg,[48] including an increase in gastrointestinal side-effects associated with either rHuIL-3 or rHuIL-6 administration.[49,50]

Considerable work has been done with rHuIL-11, which received marketing approval in the USA in 1997.[51,52] IL-11 acts late in the differentiation of megakaryocytes, affecting size and ploidy in humans.[53] Adverse events reported with rHuIL-11 include anemia, fluid retention, abnormal arrythmias, and metabolic disturbances due to necessary concomitant diuretic therapy.[52] Doses above 10 μg/kg led to increased acute-phase proteins and caused a 3–5% weight gain stemming from edema.[51] However, rHuIL-11 can be administered safely if the necessary precautions are followed, especially monitoring fluid collection, ascites, and electrolyte balance. The results of an abbreviated phase 3 study showed that rHuIL-11 prevented severe thrombocy-

topenia in patients with solid tumors or lymphoma who received chemotherapy.[54] The mean number of platelet transfusions was not significantly different (1.6 versus 2.2, $p=0.01$) between the rHuIL-11- and placebo-treated groups, nor was the time to platelet recovery to 50 or $100 \times 10^9/l$.

Other cytokines, such as granulocyte–macrophage colony-stimulating factor (GM-CSF),[55] leukemia-inhibitory factor (LIF),[42] and stem cell factor (SCF),[56] mildly affected megakaryocyte proliferation or differentiation in vitro, but did not cause a substantial increase in platelets in vivo.

In vivo studies of the Mpl ligands in mice

For a cytokine to be an endogenous physiologic regulator of thrombopoiesis, the following properties are required:[57] circulating concentrations of the serum protein increase in an acute, severe thrombocytopenic state; protein factor stimulates production of megakaryocytes and increases the release of platelet counts in both a normal and thrombocytopenic state; and circulating amounts of the factor decrease after normal numbers of platelets are attained. The effects of the Mpl ligand, MGDF, as well as of human TPO, have been evaluated in various responsive animal models, and the results suggest that the ligand for the Mpl receptor has the attributes to be considered a physiologic regulator of both megakaryocytopoiesis and thrombopoiesis.[58]

Using a platelet antiserum in mice to induce an acute, severe thrombocytopenic state, Wendling et al[59] showed that Mpl-ligand activity in serum peaked 24 hours after depletion of platelet counts. The activity remained elevated for four days until platelet counts returned to baseline, at which time the Mpl ligand concentrations decreased.

In normal mice, multiple injections of partially purified Mpl ligand increased platelet production by 20% and resulted in a 40% increase in 35S platelet incorporation.[10] Daily intraperitoneal injection of 50 ng (approximately 2.5 mg/kg) of recombinant Mpl ligand resulted in a fourfold increase in circulating platelet counts in seven days, with increases in marrow megakaryocytes (ninefold) and marrow/splenic megakaryocytic progenitors (>20-fold).[11,60] Exogenous administration of MGDF or PEG-rHuMGDF increased peripheral platelet counts after several days of administration.[28]

Taken together, the in vitro and in vivo data indicate that the ligand for the Mpl receptor is the physiologic regulator of thrombopoiesis.

Mpl ligand in murine models of thrombocytopenia

Studies of the effects of Mpl ligands have been extended to thrombocytopenic animals. Mice administered a single
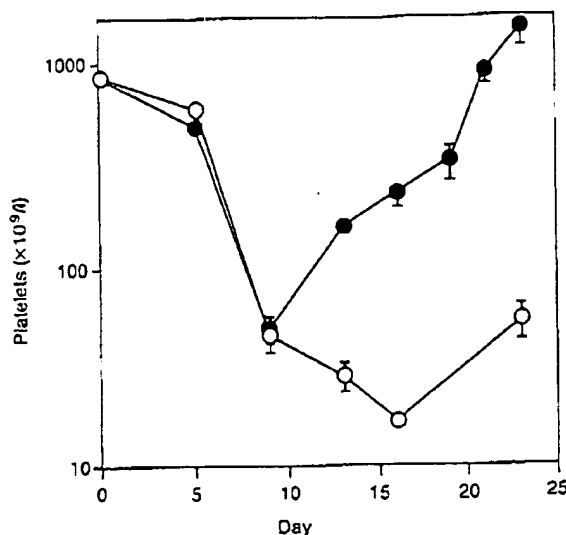
Figure 5
Effects of PEG-rHuMGDF on platelet counts in myelosuppressed mice. Mice were treated with carboplatin and irradiation. PEG-rHuMGDF was administered at 50 µg/kg/day (solid circles) or excipient (open circles) for 22 days. (From reference 28. © American Society of Hematology.)

intraperitoneal dose of 125 mg/kg carboplatin to induce thrombocytopenia were given daily intraperitoneal injections of rHuMGDF at doses of 10, 25, and 100 µg/kg/day for 12 days.[5]

Without cytokine intervention, the mice became moderately thrombocytopenic (<300 × 10⁹/l). Treatment with rHuMGDF resulted in a dose-dependent improvement in circulating platelets that was associated with increased bone marrow megakaryocytic activity.

In a model of severe thrombocytopenia originally developed by Leonard et al,[61] mice received a single intraperitoneal injection of 1.25 mg carboplatin followed four hours later with sublethal gamma-irradiation exposure of 500 rad. The myeloablated mice were administered 50 µg/kg PEG-rHuMGDF alone and in combination with 5 or 10 µg/kg rHuG-CSF. In this model, 94–100% of the animals developed lethal thrombocytopenia within 10 days. Treatment with PEG-rHuMGDF alone or in combination with recombinant murine granulocyte colony-stimulating factor (rMuG-CSF) resulted in 100% survival of animals and platelet and white blood cell recovery to normal within 22 days after initiation of cytokine treatment (Figure 5).[28] No observable toxicity was detected in the carboplatin and irradiation murine model given the combination of PEG-rHuMGDF and rMuG-CSF.[62]

Exaggerated pharmacotoxic effects of the Mpl ligand in mice

In two separate studies, high doses of PEG-rHuMGDF administered to normal mice or chronic overproduction of

endogenous Mpl ligand in genetically engineered mice resulted in extreme changes to the hematopoietic system. In the first study,[63] mice received intraperitoneal injections of 500 µg/kg/day PEG-rHuMGDF for two weeks (the therapeutic dose in thrombocytopenic mice is 50 µg/kg), and demonstrated a maximum fivefold increase in platelet counts. Thrombocytosis was associated with a transient decrease in red blood cell count and hemoglobin, megakaryocytic and myeloid hyperplasia, and a focal deposition of reticulin fibers of the marrow and splenomegaly resulting from increased splenic megakaryocytopoiesis. All of these changes were reversible after cessation of treatment. No collagen deposition or any osteosclerosis was observed in these mice.

In a second study, lethally irradiated mice received bone marrow cells containing a retroviral TPO gene.[64] This gene-transfer technique resulted in mice that chronically and constituitively overexpressed endogenous murine TPO. The mice developed osteosclerosis of the bone marrow and increased numbers of megakaryocytes in various organs, including bone marrow, spleen, liver, and lymph nodes. Chronic overexpression of TPO led to decreases in marrow hematopoiesis, including erythropoiesis and extramedullary hematopoiesis in the spleen and liver. All mice evaluated in this study had myelofibrosis and osteosclerosis, with no decrease in platelet counts. The mechanism of action of high chronic exposure of Mpl ligands (either given exogenously or genetically engineered endogenous overproduction) on the bone marrow is unknown, but may involve secondary release of growth factors by differentiating megakaryocytes or by other marrow constituents.

Microscopic examination of the bone marrow of myeloablated or normal mice treated with 50 µg/kg PEG-rHuMGDF for two weeks showed no evidence of myelofibrosis or osteosclerosis. Microscopic examination of non-hematopoietic organs in these mice showed no histopathologic changes. In hematopoietic organs (e.g., marrow, spleen, and liver) increased hematopoietic activity, including granulopoiesis and megakaryocytopoiesis, was expected. Extramedullary granulopoiesis within the mandibular and mesenteric lymph nodes was also observed, but the erythroid lineage was unaffected by cytokine treatment.[62]

Effects of MGDF and PEG-rHuMGDF in nonhuman primates

Several studies of the Mpl ligands, rHuMGDF and PEG-rHuMGDF, have been conducted in nonhuman primates. Farese et al[6] provided the first report on the effect of systemic administration of an Mpl ligand in nonhuman primates. In this study, normal rhesus monkeys were given daily subcutaneous dosages of 2.5, 25, and 250 µg/kg of rHuMGDF for 10 consecutive days (Figure 6). After six daily doses, peripheral platelet counts
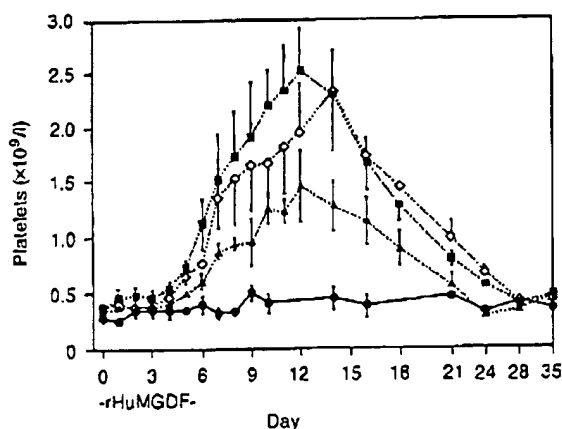
Figure 6
Effects of rHuMGDF on peripheral blood platelet count in normal nonhuman primates. rHuMGDF was administered for 4 consecutive days and human serum albumin (HSA) was administered for 10 consecutive days. Solid circles, HSA; open diamonds, 250 µg/kg PEG-rHuMGDF; solid squares, 25 µg/kg PEG-rHuMGDF; solid triangles, 2.5 µg/kg PEG-rHuMGDF. (Adapted from reference 6. © American Society of Hematology.)

increased significantly, reaching a nadir approximately 12–14 days post dose and returning to normal values by day 28. White blood cells, basophils, eosinophils, and red blood cell counts were unaffected. Clonogenic data derived from cultures of cells from humerus or iliac bone marrow aspirates showed increased Meg-CFC and GEMM-CFC colonies on days 3, 10, 16, and 28. The number of early progenitors for white cell and erythroid lineages, GM-CFC and E-BFC, respectively, remained unchanged during the administration of rHuMGDF.

These data suggest that the rhesus monkey is a responsive model to the systemic administration of rHuMGDF and that administration of rHuMGDF can increase progenitor cell numbers in the bone marrow without affecting the erythroid or myeloid lineages. In the irradiated rhesus monkey model,[65] administration of PEG-rHuMGDF reduced the severity and duration of thrombocytopenia.

The aforementioned animal studies further support the use of Mpl ligands, such as PEG-rHuMGDF, in the treatment of radiotherapy- and/or chemotherapy-induced thrombocytopenia in humans. In a baboon model, however, the choice of chemotherapy and the severity of the thrombocytopenia may play a role in the ability of a cytokine to promote hematopoietic recovery. Hepsulfan-induced thrombocytopenia has different kinetics than irradiation-induced thrombocytopenia;[66,67] hepsulfan-induced thrombocytopenia is less severe, occurs later, and has a longer duration. In a baboon model, administration of PEG-rHuMGDF maintained platelet counts at or above prechemotherapy values without significantly affecting the associated neutropenia and anemia.[67] Coadministration of PEG-rHuMGDF and rHuG-CSF in a rhesus monkey model of hepsulfan-induced myelosup-

pression showed complete abolition of thrombocytopenia.[68]

Effects of Mpl ligand administration on platelet function and physiology

The potential effects of the exogenous administration of a platelet stimulator on platelet physiology are of obvious concern and importance. The process of maintaining hemostasis of the circulatory system involves the formation of a clot induced by platelets. Immediately after endothelial blood vessel wall damage or perturbation, circulating platelets adhere to the vessel wall. This adhesion causes the platelets to change shape and release synthesized thromboxane, ADP calcium, and platelet-activating factor, which further cause the platelets to aggregate and form a plug to close the rupture. Additional coagulatory cascade steps are activated to stabilize the clot with fibrin.

In an experimental baboon model of platelet deposition and fibrin accumulation developed by Harker et al,[69] exogenous administration of rHuMGDF or PEG-rHuMGDF resulted in increased platelet counts, with a concomitant increase in megakaryocytes. Platelet deposition increased in proportion to increased platelet number, with no fibrin accumulation. These studies suggest that rHuMGDF can enhance platelet disposition with minimal thrombotic effects.

In a series of ex vivo studies of platelet aggregometry, Toombs et al[70] reported that rHuMGDF alone had no effect on platelet aggregation. In the presence of platelet agonists, preincubation of rHuMGDF caused a moderate enhancement in platelet sensitivity.

Since the Mpl receptor is located on platelets, its role in stimulating platelet activation has been studied. At a concentration of 50 ng/ml, TPO was shown to stimulate platelets in the presence and absence of exogenous fibrinogen, using protein phosphorylation as an endpoint. In addition, TPO plus fibrinogen was shown to induce a concentration-dependent primary and secondary aggregation of washed platelets.[71]

Clinical applications of the Mpl ligand, PEG-rHuMGDF

The discovery and clinical use of recombinant human hematopoietic growth factors, such as rHuG-CSF as a restorative agent in the treatment of myelotoxicity, has been a major advance in cancer chemotherapy. The use of higher doses of antineoplastic agents is now possible when coupled with rHuG-CSF; however, the higher doses of antineoplastic agents also create the potential for hemorrhagic complications associated with thrombocytopenia.

Clinical studies in an oncology setting

Basser et al[7] were the first to publish the results of studies of Mpl ligands in humans. In a phase 1 study, 17 patients with cancer were administered various doses of PEG-rHuMGDF or placebo before chemotherapy for up to 10 days. A dose-dependent increase in platelet count and in the number of bone marrow megakaryocytes occurred at doses of 0.3 and 1.0 μg/kg/day. In another phase 1 study, 50 patients with advanced lung cancer received PEG-rHuMGDF in a randomized, double-blind, placebo-controlled, dose-escalation study.[9] Patients receiving PEG-rHuMGDF had higher nadir platelet counts (188×10^9/l) than those receiving placebo (111×10^9/l). The PEG-rHuMGDF-treated patients had an earlier recovery to baseline platelet values (14 days) than patients who received placebo (21 days) ($p < 0.005$). This study was the first to demonstrate that PEG-rHuMGDF reduces the severity of thrombocytopenia in patients receiving cancer chemotherapy.

In another phase 1, placebo-controlled study, 40 patients with advanced cancer received carboplatin (600 mg/m^2) and cyclophosphamide (1200 mg/m^2).[8] Filgrastim (r-metHuG-CSF) was given after chemotherapy for support of myeloid recovery. Patients were randomized to receive either PEG-rHuMGDF (0.3–5.0 μg/kg/day) or placebo after chemotherapy. Recovery to baseline platelet counts was faster in PEG-rHuMGDF-treated patients (17 days) than in patients receiving placebo (22 days). In all of these studies, PEG-rHuMGDF was well tolerated and adverse events were similar in the patients receiving placebo and in PEG-rHuMGDF-treated patients.

O'Malley et al[72] studied platelet function in a phase 1 study of PEG-rHuMGDF. Platelet counts increased threefold after 16 days of administration of 0.3 or 1.0 μg/kg/day PEG-rHuMGDF. The platelets formed by PEG-rHuMGDF stimulation showed normal aggregation responses when stimulated with a wide range of agonists (Figure 7), and showed no change in expression of P-selectin or GPIIb/IIIa fibrinogen-binding sites. Reticulated platelet counts, used as a measure of new platelet production, increased after three days of treatment with PEG-rHuMGDF, and plasma glycocalicin, used as a measure of platelet turnover, increased in proportion to platelet counts.

Other potential uses

High-dose chemotherapy for the treatment of lymphoma requires bone marrow transplantation or the transplantation of cytokine-mobilized peripheral blood progenitor cells (PBPC). A reduction in the number of platelet transfusions required and earlier release from hospital has been reported for patients with lymphoma who were administered PBPC.[73] A further reduction in the number of platelet transfusions might be achievable by using rHuMGDF to stimulate new platelet production after engraftment, or by pretreating progenitor cells with rHuMGDF to expand the colony to mature megakaryocytes and megakaryocyte progenitor cells for transplantation, a technique known as ex vivo expansion (see below)

Other blood diseases, such as chronic idiopathic thrombocytopenic purpura, are autoimmune diseases characterized by a persistent thrombocytopenia with a mean platelet count of 9 (±2) $\times 10^9$/l or more.[74] Thrombocytopenia in this disease setting is persistent owing to detectable antiplatelet autoantibodies that cause platelet destruction by the reticuloendothelial system. George[75] and Kosugi et al[24] reported that the circulating concentrations of endogenous TPO were not greatly increased compared with values in normal patients.

Preleukemic myelodysplastic syndromes (MDS) are hematopoietic stem cell abnormalities in which lack of maturation of progenitor cells leads to an increased risk of leukemic transformation. Bouscary et al[76] reported on c-*mpl* expression in patients with MDS. Their results indicated that some patients with refractory anemia had c-*mpl* expression and dysmegakaryopoiesis. A subgroup may benefit from PEG-rHuMGDF treatment to restore platelet production.

Potential use in transfusion medicine

For almost a quarter of a century, platelet transfusion therapy has been used successfully to prevent death from hemorrhage associated with chronic thrombocytopenia.
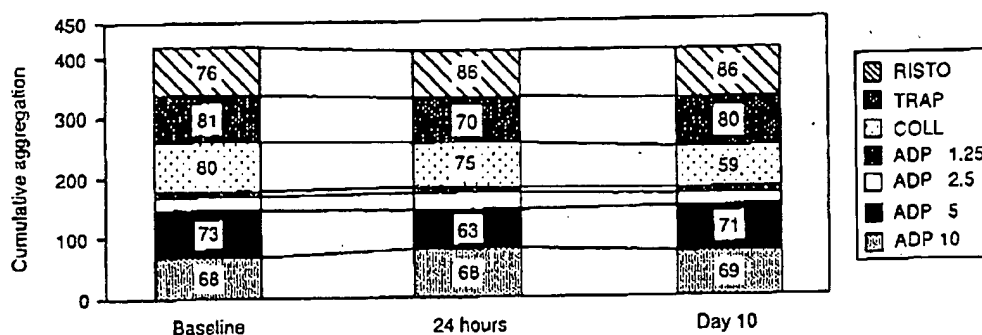
Figure 7
Platelet aggregation was for an individual patient treated with 0.3 μg/kg/day PEG-rHuMGDF. RISTO, ristocetin; TRAP, thrombin-receptor agonist peptide; COLL, collagen. Percentage of aggregation given only if greater than 20%. (Adapted from reference 72. © American Society of Hematology.)

The potential risks with platelet infusion include transmission of fatal disease, 'infection', immune response in recipients making additional transfusions ineffectual, and, in rare cases, the onset of graft-versus-host disease.

Transfusion medicine may have potential for Mpl ligands, which could be used to stimulate peripheral blood progenitor cells, some of which have TPO receptors.[77] Whether recombinant Mpl ligands can stimulate PBPC to any extent is unknown, but studies show that PEG-rHuMGDF can increase bone marrow CFC-M and CFC-GEMM,[6] and some mobilizing activity has been detected in patients.[8]

The administration of recombinant Mpl ligands to patients and normal volunteers produced a predictable increase in platelets starting on day 7,[58,78,79] and this increase could allow the routine collection of large numbers of platelets from a single plateletpheresis.[79] This collection may contain younger platelets, which might be expected to have a longer circulating lifespan after transfusion compared with platelets from traditional plateletpheresis. However, in this study, normal volunteers developed neutralizing antibodies, which necessitated the cancellation of further studies for this indication and in this population.

Choi et al[4] described a method that may make it possible to use recombinant Mpl ligands to produce platelets by ex vivo expansion. These morphologically and physio-logically normal platelets can be produced by treating cultures of CD34+ cells with PEG-rHuMGDF, thereby avoiding the development of neutralizing antibodies. However, much work is still required to optimize ex vivo expansion technique. Problems with current methods of ex vivo expansion include homing of expanded cell populations and aging of some populations of expanded cells.[50] Many regulatory issues also remain unresolved in Good Manufacturing Processes, which now are largely unregulated.

Conclusions

The discovery of the Mpl receptor was a breakthrough in the understanding of megakaryocyte development and platelet formation. Several cytokines have been shown to influence platelet production; however, the ligand for the Mpl receptor has been shown to be the primary regulator of megakaryocytopoeisis and thrombopoiesis. The application of exogenous new non-immunogenic recombinant Mpl ligands will hopefully be demonstrated in randomized clinical trials. The application of this therapy in the mobilization of CD34+ stem cells, ex vivo expansion techniques, and use in platelet abnormalities should be explored.

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